Synthesis of phosphorylated fragments of *Streptococcus pneumoniae* type 19F capsular polysaccharide

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Serotype 19F is one of the most virulent of the *Streptococcus pneumoniae* serotypes, causing meningitis, otitis media, septicemia and pneumonia. Effective protection against infection is mediated by antibodies directed against its capsular polysaccharide (CPS), composed of the trisaccharide repeating units (⟶4-β-D-Man*p*NAc-(1⟶4)-α-D-Glc*p*-(1⟶2)-α-L-Rha*p*-1⟶) bridged by phosphate diesters. Unfortunately, CPSs are typical T-independent type 2 immunogens. Therefore, the conjugation of putative saccharide haptens to a protein carrier, which invokes T-cell involvement, has proved to be extremely effective in enhancing the immune response. In a project aimed at ascertaining the role played by the different structural factors in the immunogenicity of new vaccines against *Streptococcus pneumoniae* type 19F, we planned the synthesis of a family of trisaccharide repeating unit derivatives where the phosphate group is a) absent; b) linked only to the rhamnose unit; or c) linked only to the *N*-acetylmannosamine unit. Compound of type a) and c) also bear an appropriate spacer for conjugation to the protein carrier. Moreover, the synthesis of a dimer of the CPS repeating unit, in which a phosphate diester bridges two repeating units, was carried out.

Introduction

Vaccination is the most cost-effective means to protect populations against infectious diseases caused by pathogenic bacteria. Amongst these *Streptococcus pneumoniae*[1] is one of the major causes of death and disability throughout the world,[2] causing meningitis, otitis, septicemia and pneumonia, both in developed and in developing countries.[3] *Streptococcus pneumoniae* belongs to the class of encapsulated bacteria that are enveloped by a carbohydrate coat (capsule). This exerts a protective function against the host's immune defense. The capsular polysaccharides (CPSs) of the bacteria, which define the different serotypes, are the key determinants of virulence, and the resistance to infection from encapsulated bacteria is mediated by the presence of antibodies directed against their CPSs. Ninety serotypes[4] of *S. pneumoniae* have been defined, associated with different diseases, age groups and geographical areas. Therefore, the design of a "universal" vaccine is extremely complex. Nevertheless, five polyvalent vaccines against these bacteria, based on a mixture of some of their CPSs, have been successfully developed.[3a] Unfortunately, as CPSs are typical T-independent type 2 immunogens, the antibody response induced by their administration drops in a few years in adults, and children under two years of age are unresponsive. The immune response can be improved by chemical conjugation of the saccharide vaccines to a protein carrier (typically, CRM197, a variant of diphtheria toxin), which invokes T-cell involvement.[5] Glycoconjugate vaccines of this kind have proved to be extremely effective against *Haemophilus influenzae* type B infections.

Rational development of glycoconjugate vaccines is hampered by the lack of understanding of their fundamental science—including the molecular mechanisms by which T-cell dependent immune responses against the saccharide are induced. Despite suggestions in the literature[6] that the immune responses induced by glycoconjugate vaccines can be tailored to optimize protection and immunogenicity, no systematic study has been carried out to elucidate the principles on which this would be based. Such knowledge would greatly aid the rapid and rational development of novel vaccines against important diseases and the optimization of the immunogenicity of existing vaccines.

We are currently involved in a project aimed at a rational development of glycoconjugate vaccines by elucidation of their mechanism of action and the development of standardized synthetic methodologies The whole project can contribute to determining the role played by different structural factors in the activity of new glycoconjugate vaccines. In particular, synthetic oligoglycoconjugates can be useful in establishing the chain length, the number of incorporated carbohydrate residues and other structural features that are required for optimal immunological activity. Furthermore, they can overcome the problems of product heterogeneity and biological contamination associated with the use of native carbohydrate material. Finally, they allow a full characterisation and immunological evaluation of the synthesised compounds. This will aid the development of new vaccines and their acceptance onto the market by licensing authorities.

The general target is a glycoconjugate molecule in which a saccharidic portion is covalently linked to a carrier protein through a spacer. We focused our attention on serotype 19F of *S. pneumoniae*, whose CPS is composed of trisaccharide repeating units (⟶4-β-D-Man*p*NAc-(1⟶4)-α-D-Glc*p*-(1⟶2)-α-L-Rha*p*-1⟶) linked through phosphodiester bridges. Syntheses of the saccharidic part of the 19F repeating unit have already been reported by us[7] and others.[8]

In order to optimize the design of the saccharidic part we needed to study the role of the phosphate group and the linkage between the saccharidic part and the carrier protein. An important issue to be addressed is the role of the phosphate

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group, as charged groups are often important epitopes for pneumococcal PSs.[9] In the 19F CPS the phosphate groups are linked to the rhamnose of one unit and to the *N*-acetylmannosamine of the subsequent unit, and thus it is of interest to assess the structure–activity relationship when the phosphate is a) absent; b) linked only to the rhamnose unit; c) linked only to the *N*-acetylmannosamine unit; d) bridging two repeating units.

A second question involves evaluation of the influence of the configuration of the linkage between the saccharidic part and the carrier protein, as this could affect the presentation of the antigen.

To look into these problems, in the present paper we describe the synthesis of a family of trisaccharides (**4**, **11α**, **11β**, **12α**, **12β**, **15**) covering all of the above aspects. In compound **4** the phosphate group is α-linked to the anomeric position of the rhamnose unit. Compounds **11α** and **11β** bear a 4″-*O*-phosphate group and have a linker at the anomeric position of the rhamnose, which is α-linked in **11α**, and β-linked in **11β**. Compounds **12α** and **12β** correspond, respectively, to **11α** and **11β** in which the phosphate group is absent. Finally, in dimer **15**, bearing only the α-oriented spacer arm, the phosphate group is linked both to the rhamnose and to the *N*-acetylmannosamine units.

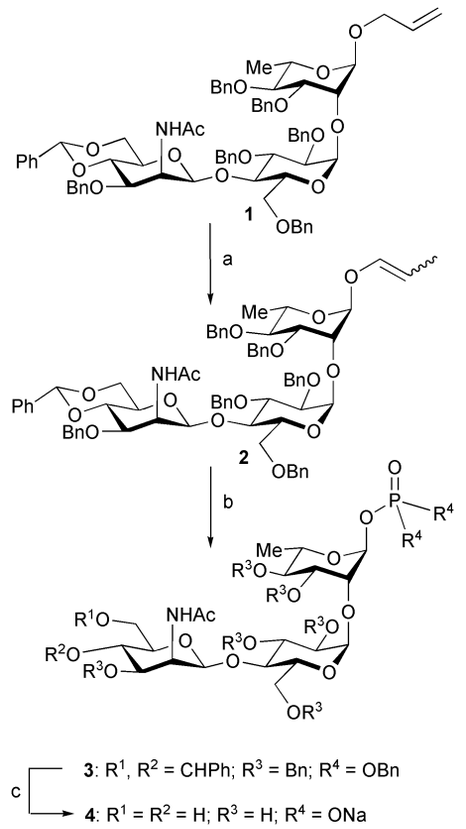
It is planned to test the above compounds as inhibitors in an ELISA experiment in order to assess their ability to inhibit the binding of the CPS to anti-Pn19F antiserum. This will indicate which residues are most important for oligosaccharide–antibody binding, although not those factors which may be predictive of the induction of productive immunity by the final oligosaccharide conjugate.

Results and discussion

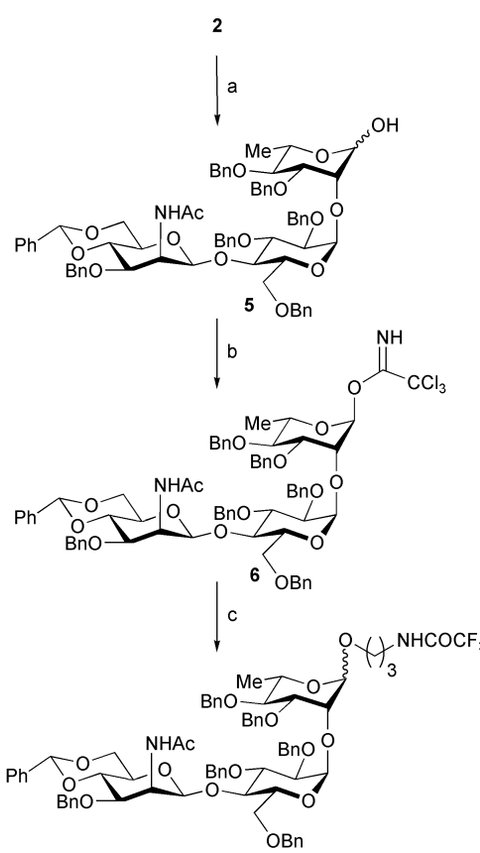
The strategically protected trisaccharide **1**, corresponding to the repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 19F, has been obtained on a multigram scale *via* a procedure previously described by us[7] and employed for the synthesis of compounds **4**, **11α**, **11β**, **12α**, **12β**, **15**.

The 1-*O*-allyl group of compound **1** was isomerized[10] into the corresponding 1-*O*-propenyl glycoside **2**, which was directly used as a glycosyl donor[11] with dibenzyl phosphate as a nucleophile. The glycosylation was promoted by NIS and TMSOTf and afforded the glycosyl 1-*O*-dibenzyl phosphate **3** as the pure α anomer (72%). Removal of the protecting groups was achieved by hydrogenolysis over $Pd(OH)_2$ and gave trisaccharide **4** as its disodium salt (Scheme 1).

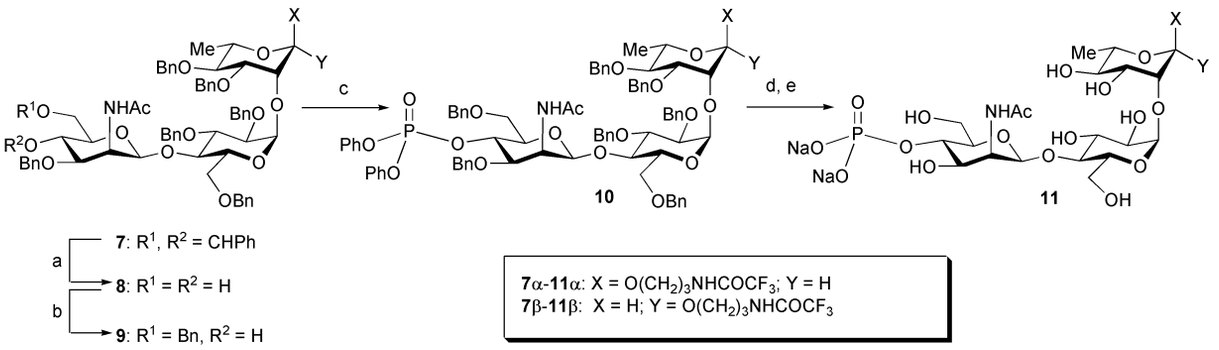
On the other hand, complete removal of the allyl group from **1** allowed the introduction of the *N*-trifluoroacetamidopropyl group as an *N*-protected spacer arm for conjugation to protein carriers. Thus, hydrolysis of **2** with iodine in moist tetrahydrofuran[12] afforded the known[7b] trisaccharide **5**, which was converted into the trichloroacetimidate **6**[13] by treatment with trichloroacetonitrile in CH_2Cl_2 in the presence of a catalytic amount of DBU (Scheme 2). Despite many attempts, performed employing different Lewis acids as well as different reaction conditions, the glycosylation with 3-(trifluoroacetamido)-propan-1-ol (purchased by Fluka) invariably led to α–β mixtures of the glycoside **7**. The best result was achieved in dry Et_2O at room temperature using $BF_3{\cdot}OEt_2$ as the acidic promoter (98% yield of a *ca.* 5 : 2, α : β mixture). Nevertheless, we took advantage of this result to explore the influence on the antigenic presentation of the configuration of the linkage joining the saccharidic moiety to the protein carrier. The two anomers of glycoside **7** were carefully separated by medium pressure chromatography giving pure **7α** and **7β** in 69 and 27% yield, respectively. The benzylidene acetal of **7α** was hydrolysed with 90% aq. trifluoroacetic acid in CH_2Cl_2 and the resulting diol **8α** was regioselectively 6c-*O*-benzylated *via* the correspond-



Scheme 1 *Reagents and conditions*: (a) EtOH, Wilkinson's catalyst, DBU, quantitative; (b) NIS, dibenzyl phosphate, CH_2Cl_2–Et_2O, ms 4 Å, 72%; (c) H_2, $Pd(OH)_2$, EtOAc–MeOH–H_2O + HOAc, then Dowex 50W-X8 (Na^+ form), 95%.



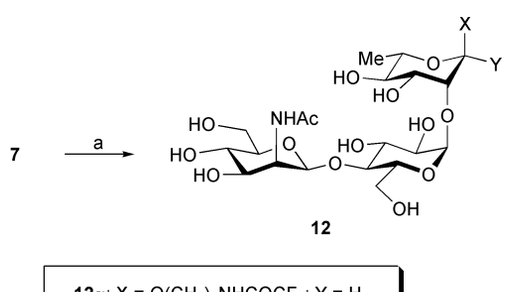
Scheme 2 *Reagents and conditions*: (a) I_2, THF–H_2O,[11] quantitative; (b) Cl_3CCN, DBU, CH_2Cl_2, 96%; (c) $HO(CH_2)_3NHCOCF_3$, $BF_3{\cdot}OEt_2$, Et_2O, rt, 98%, α : β = 5 : 2.



Scheme 3 *Reagents and conditions*: (a) 90% aq. CF_3COOH, CH_2Cl_2, 0 °C, quantitative; (b) Bu_2SnO, toluene, then BnBr, tetrabutylammonium iodide, **9α**: 78%, **9β**: 76%; (c) diphenyl phosphorochloridate, DIPEA, DMAP, CH_2Cl_2, **10α**: 49%, **10β**: 61%; (d) H_2, $Pd(OH)_2$, MeOH–H_2O; (e) H_2 (5 bar), PtO_2, H_2O, then Dowex 50W-X8 (Na^+ form), **11α**: 71%, **11β**: 81%.

ing stannylene acetal to afford compound **9α** in 78% overall yield (Scheme 3). 4c-*O*-Phosphorylation of **9α** was carried out with diphenyl phosphorochloridate in CH_2Cl_2 in the presence of diisopropylethylamine and 4-*N*,*N*-dimethylaminopyridine (DMAP)[14] providing trisaccharide **10α** in 49% yield. Complete removal of the protecting groups was accomplished in two steps. First, the benzyl ethers were cleaved by hydrogenolysis over $Pd(OH)_2$ in a methanol–water mixture. Thereafter, the phenyl esters were removed by hydrogenation (5 bar) over PtO_2 in water. After filtration over a Celite pad, the compound was eluted with water through a column filled with Dowex 50W-X8 resin (Na^+ form) and lyophilised, giving 4c-*O*-phosphorylated trisaccharide **11α** in 71% overall yield. Likewise, through the same reaction sequence applied for the synthesis of **11α**, trisaccharide **11β** was obtained from **7β** (Scheme 3).

Moreover, to evaluate the influence on the immunological activity of the 4c-*O*-phosphorylation 4c-OH trisaccharides **12α** and **12β** were synthesised by hydrogenation over $Pd(OH)_2$ of **7α** and **7β**, respectively (Scheme 4).



Scheme 4 *Reagents and conditions*: (a) H_2, $Pd(OH)_2$, MeOH–H_2O, 95%.

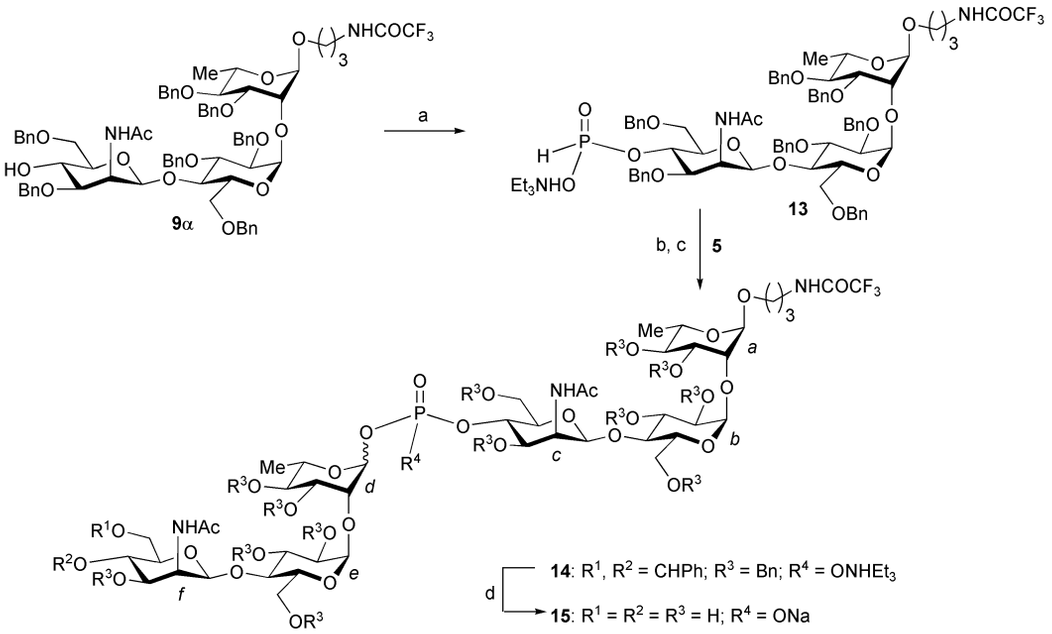
Finally, the synthesis of the hexasaccharide **15** was attempted. From the other methods available for the synthesis of glycosylphosphodiester bridges,[15] we chose the H-phosphonate approach.[16] Within this context, in principle two different procedures can be adopted, the first one consisting of the synthesis of the anomeric H-phosphonate monoester[16,17] of trisaccharide **5** followed by coupling with compound **9α**. Disappointingly, we invariably obtained a *ca.* 7 : 3, α : β mixture of anomers, using both the *in situ* formed triimidazolylphosphine[16,18] and 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one[19] in the formation of the H-phosphonate monoester on **5**. Gratifyingly, the formation of the H-phosphonate monoester on trisaccharide **9α**,[20] followed by the coupling with compound **5**, allowed better stereocontrol—even if not absolute, as it turned out at the end of our attempts. Hydrogen phosphonate **13** was obtained by the treatment of **9α** with a slight excess of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in a mixture of dry acetonitrile and pyridine at room temperature (Scheme 5). After work up and purification by filtration over a short column of silica gel, **13** was recovered as its triethylammonium salt in almost quantitative yield. The coupling of **13** with **5** was carried out using pivaloyl chloride as a condensing agent in pyridine and followed by *in situ* oxidation with iodine in pyridine–water[18] to give phosphodiester-linked hexasaccharide **14** its triethylammonium salt in 61% overall yield (Scheme 5). The ^1H NMR spectrum of compound **14** showed extensive overlap of the signals, which did not allow its complete spectroscopic characterization. Only after removal of the protecting groups by hydrogenation over $Pd(OH)_2$ on charcoal, followed by filtration over Dowex 50W-X8 resin (Na^+ form), was hexasaccharide **15** recovered as a white glass and fully characterized by NMR spectroscopy. Moreover, HPLC analysis showed the presence of a 9 : 1, α : β mixture of diastereoisomers of **15**, differing in the configuration of the anomeric linkage of the inner rhamnose unit (unit *d*), as deduced from the NMR spectra.

Conclusions

We have described the synthesis of a new family of *Streptococcus pneumoniae* 19F CPS fragments, differing in the position of the phosphate group or in the anomeric configuration of a spacer suitable for conjugation to a carrier protein. These compounds allow the assessment of the role of the phosphate group in the immunological activity of the fragments. Moreover, the influence of the configuration of the linkage between the saccharidic part and the carrier protein linkage in presenting the antigen can be studied starting from compounds **12α**,**β**.

Experimental

TLC was carried out on Merck silica-gel 60 F_{254} plates (0.25 mm thickness), and spots were visualized by spraying with a solution containing H_2SO_4 (31 cm^3), ammonium molybdate (21 g) and $Ce(SO_4)_2$ (1 g) in 500 cm^3 of water, followed by heating at 110 °C for 5 min. Column chromatography was performed by the flash procedure using Merck silica-gel 60 (230–400 mesh). Melting points were determined with Büchi apparatus and are not corrected. Optical rotations were measured at room temperature (25 °C) with a Perkin-Elmer 241 polarimeter and $[\alpha]_D$ values are given in units of in 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded at 30 °C on Bruker AC 300, Varian Unity 500 and ™Varian XL 200 Gemini spectrometers. In the description of the NMR spectra a, b, c, d, e and f refer to the monosaccharide units in the oligosaccharides (a = reducing end), and coupling constants are given in Hz. Signals corresponding to aromatic carbons were omitted from the ^{13}C spectra. Analytical HPLCs were carried out on a system consisting of a SpectraPhysics P2000 gradient pump, a SpectraPhysics 100 UV/vis detector and a SpectraPhysics SP4270 integrator, with a 20 cm × 4.6 mm HyperCarb column (5 μm)



Scheme 5 *Reagents and conditions*: (a) 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one, py, CH_3CN, rt; (b) **5**, PivCl, py, rt; (c) I_2, py–H_2O 19 : 1, rt, 61% from **9α**; (d) H_2, $Pd(OH)_2$, MeOH–H_2O, then Dowex 50W-X8 (Na^+ form), 96%.

(where not indicated). Elemental analyses were performed using the Carlo Erba elemental analyzer 1108.

Prop-1-enyl (2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 2

To a solution of **1** (200 mg, 0.17 mmol) in dry THF (30 cm^3) was added a catalytic amount of (cycloocta-1,5-diene)bis-(methyldiphenylphosphine)iridium hexafluorophosphate. The stirred solution was degassed by freezing the solvent and allowing it to warm to room temperature under vacuum and then placed under H_2 to activate the catalyst (the slightly red suspension became pale yellow). After 2 min, the solution was degassed once more and stirred at room temperature under N_2. After 2 h, TLC analysis showed a complete conversion of the allyl ether into the 1-*O*-propenyl ether. The reaction mixture was concentrated to dryness and the resulting yellow oil was filtered on a short column of silica gel (petroleum ether–EtOAc 7 : 3) to remove the catalyst. Crude **2** (180 mg) was used directly in the next step without further characterization.

(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl dibenzyl phosphate 3

Propenyl glycoside **2** (170 mg, 0.14 mmol) was dissolved in CH_2Cl_2–Et_2O 2 : 1 (6 cm^3) containing 4 Å molecular sieves (100 mg). The suspension was stirred under Ar for 1 h, then cooled at −40 °C. NIS (150 mg, 0.6 mmol) and dibenzyl phosphate (160 mg, 0.57 mmol) were added, followed by a 0.1 M solution of TMSOTf in CH_2Cl_2 (14 mm^3, 0.0014 mmol). After 30 min the reaction mixture was neutralised with Et_3N, filtered over a Celite pad and the solvent evaporated. The crude residue was first purified by flash chromatography (petroleum ether–EtOAc 6 : 4). Final purification was achieved by medium pressure chromatography (same eluent) to give **3** (143 mg, 72%) as a white solid (Found: C, 72.5; H, 5.7; N, 9.1. $C_{93}H_{88}NO_{18}P$ requires C, 72.6; H, 5.75; N, 9.1%); mp 115–116 °C; $[\alpha]_D$ +6.7 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.31 (3 H, d, $J_{6a,5a}$ 6.0, H-6a), 1.80 (3 H, s, CH_3CO), 3.01–3.10 (1 H, m, H-5c), 3.18 (1 H, br d, $J_{6b,6'b}$ 10.9, H-6b), 3.29–3.34 (2 H, m, H-6′b, H-3c), 3.49–3.63 (4 H, m, H-4a, H-2b, H-5b, H-6c), 3.76–4.08 (7 H, m, H-2a, H-3a, H-5a, H-3b, H-4b, H-4c, H-6c), 4.26 (1 H, d, *J* 11.9, CH*H*Ph), 4.45–5.04 (18 H, m, 7 × CH_2Ph, CH*H*Ph, H-1b, H-1c, H-2c), 5.45 (1 H, s, C*H*Ph), 5.55 (1 H, d, *J* 9.3, NHAc), 5.68 (1 H, br d, $J_{1a,2a}$ 5.9, H-1a) and 7.05–7.50 (45 H, m, 9 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 17.8 (q, C-6a), 23.1 (q, *C*H_3CO), 50.5 (d, C-3c), 67.9, 68.6, 69.4, 71.1, 72.1, 72.7, 73.3, 74.6, 75.2 (9 × t, C-6b, C-6c, 8 × *C*H_2Ph), 67.1, 69.9, 70.4, 74.4, 75.6, 75.9, 77.7, 78.4, 79.3, 80.3 (10 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 96.1 (d, $J_{C,P}$ 8.7, C-1a), 96.9, 99.8 (2 × d, C-1b, C-1c), 101.6 (d, *C*HPh) and 177.7 (s, *C*ONH); δ_P(200 MHz; $CDCl_3$, D_2O–H_3PO_4–H_2O = 90 : 8.5 : 1.5) −2.39.

(2-Acetamido-2-deoxy-β-D-mannopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→2)-α-L-rhamnopyranosyl dihydrogen phosphate disodium salt 4

Trisaccharide **3** (32 mg, 0.022 mmol) was dissolved in EtOAc–MeOH–H_2O 1 : 1 : 1 (6 cm^3, containing 1 drop of HOAc) and hydrogenolyzed at 10 bar over $Pd(OH)_2$ (40 mg) for 16 h. The mixture was filtered over a Celite pad and the filtrate was concentrated, then eluted with water through a column filled with Dowex 50W-X8 resin (Na^+ form). Lyophilization gave trisaccharide **4** (13 mg, 95%) as a white foam (Found: C, 36.85; H, 5.25; N, 2.10. $C_{20}H_{34}NO_{18}PNa_2$ requires C, 36.80; H, 5.24; N, 2.14%); $[\alpha]_D$ +18.6 (*c* 0.5 in H_2O); the NMR spectrum of compound **4** is reported in Table 1.

O-(2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α,β-L-rhamnopyranosyl trichloroacetimidate 6

Compound **5** (872 mg, 0.753 mmol), obtained from trisaccharide **2** as previously described,[7b] was dissolved in dry CH_2Cl_2 (30 cm^3) under a nitrogen atmosphere. After cooling to 0 °C, CCl_3CN (529 mm^3, 5.27 mmol) and DBU (11 mm^3, 0.075 mmol) were added under N_2 and the mixture was stirred for 15 min at 0 °C. The mixture was concentrated and the residue was purified by flash chromatography (petroleum ether–EtOAc 7 : 3 + 1% Et_3N) to give **6** (only α anomer, 942 mg, 96%) isolated as a glass (Found: C, 65.40; H, 5.90; N, 2.10. $C_{71}H_{75}N_2O_{15}Cl_3$ requires C, 65.46; H, 5.80; N, 2.15%); $[\alpha]_D$ +3.0 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.39 (3 H, d, $J_{6a,5a}$ 6.0, H-6a), 1.79 (3 H, s, CH_3CO), 3.06 (1 H, dt, $J_{5c,6c}$ 9.8, $J_{5c,4c}$ 9.8, $J_{5c,6'c}$ 4.9,

Table 1 ^{1}H NMR (D_2O, 500 MHz, 303 K) and ^{13}C NMR (D_2O, 125.72 MHz, 303 K) of compound **4**[a]

Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6′	NAc
a	5.37	3.97	[3.95]	3.45	[3.90]	1.27		
	93.8	70.2	78.6	73.1	70.3	17.8		
b	5.04	3.56	3.87	3.67	4.07	3.74	3.74	
	98.8	72.2	72.2	79.7	71.3	60.8		
c	7.87	4.53	3.80	3.51	3.43	3.91	3.80	2.05
	100.2	54.2	73.0	67.5	77.5	61.3		23.0

[a] Chemical shifts referenced to highfield *N*-acetyl at 2.050 and 23.00 ppm. [ ] = uncertain assignment.

H-5c), 3.29 (1 H, br d, $J_{6'b,6b}$ 10.8, H-6′b), 3.34 (1 H, dd, $J_{2c,3c}$ 4.2, $J_{3c,4c}$ 9.8, H-3c), 3.44 (1 H, br d, $J_{6b,6'b}$ 10.8, H-6b), 3.50–3.69 (5 H, m, H-3a, H-4a, H-2b, H-5b, H-4c), 3.83–4.10 (5 H, m, H-5a, H-3b, H-4b, H-6c, H-6′c), 4.13 (1 H, br s, H-2a), 4.30 (1 H, d, *J* 11.9, C*H*HPh), 4.48 (1 H, d, *J* 12.9, CH*H*Ph), 4.50 (1 H, br s, H-1c), 4.58–4.74 (8 H, m, H-2c, 3 × CH*H*Ph, 2 × CH_2Ph), 4.81 (1 H, d, *J* 11.8, CH*H*Ph), 4.96–5.01 (3 H, m, 2 × C*H*HPh, H-1b), 5.47 (1 H, s, C*H*Ph), 5.51 (1 H, d, *J* 9.6, NHAc), 6.28 (1 H, br s, H-1a), 7.05–7.55 (35 H, m, 7 × Ph) and 8.53 (1 H, s, NH).

3-Trifluoroacetamidopropyl (2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α,β-L-rhamnopyranoside 7α, 7β

Compound **6** (343 mg, 0.26 mmol) and 3-(trifluoroacetamido)-propan-1-ol (608 mg, 3.55 mmol) were dissolved in dry Et_2O at rt, then $BF_3{\cdot}Et_2O$ (66 mm^3, 0.52 mmol) was added dropwise under N_2 with stirring for 10 min. The reaction mixture was quenched with Et_3N and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether–EtOAc 6 : 4) to give **7** as a mixture of anomers (338 mg, 98%). The anomers were separated by medium pressure chromatography (petroleum ether–EtOAc 65 : 35). First, the β anomer **7β** (94 mg) was obtained as a glass (Found: C, 67.70; H, 6.25; N, 2.10. $C_{74}H_{81}N_2O_{16}F_3$ requires C, 67.77; H, 6.23; N, 2.14%); $[\alpha]_D$ +30.6 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.38 (3 H, d, $J_{6a,5a}$ 6.0, H-6a), 1.72 (3 H, s, CH_3CO), 1.70–1.80 (2 H, m, OCH$_2$CH_2CH$_2$NHCOCF$_3$), 1.99–3.14 (2 H, m, H-5c, O(CH$_2$)$_2$CH*H*NHCOCF$_3$), 3.23–3.64 (11 H, m, H-3a, H-4a, H-5a, H-2b, H-6b, H-6′b, H-3c, H-4c, H-6′c, O(CH$_2$)$_2$C*H*HNHCOCF$_3$, OCH*H*(CH$_2$)$_2$NHCOCF$_3$), 3.74–3.82 (1 H, m, OC*H*H (CH$_2$)$_2$NHCOCF$_3$), 3.92 (1 H, t, *J* 9.2, H-4b or H-3b), 3.99 (1 H, t, *J* 9.2, H-4b or H-3b), 4.07 (1 H, dd, $J_{6c,6'c}$ 10.4, $J_{6c,5c}$ 4.9, H-6c), 4.21–4.26 (3 H, m, H-2a, H-5b, CH*H*Ph), 4.39 (1 H, s, H-1a), 4.45 (1 H, br s, H-1c), 4.47–4.74 (8 H, m, 7 × C*H*HPh, H-2c), 4.80 (1 H, d, *J* 11.7, C*H*HPh), 4.83 (1 H, d, *J* 11.7, CH*H*Ph), 4.97 (1 H, d, *J* 11.7, C*H*HPh), 4.99 (1 H, d, *J* 10.9, CH*H*Ph), 5.43 (1 H, d, $J_{1b,2b}$ 3.7, H-1b), 5.47 (1 H, s, C*H*Ph), 5.49 (1 H, d, *J* 9.7, NHAc), 6.99 (1 H, br s, NHCOCF$_3$) and 7.08–7.52 (35 H, m, 7 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 17.8 (q, C-6a), 23.2 (q, *C*H$_3$CO), 28.6 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 37.2 (t, O(CH$_2$)$_2$*C*H$_2$-NHCOCF$_3$), 50.6 (d, C-2c), 67.9, 68.1, 68.7, 71.2, 71.8, 72.2, 73.4, 74.4, 75.4 (9 × t, C-6b, C-6c, 6 × *C*H$_2$Ph, O*C*H$_2$-(CH$_2$)$_2$NHCOCF$_3$), 67.2, 69.6, 72.3, 73.8, 75.8, 75.9, 78.5, 79.2, 79.6, 80.0, 81.2 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.6, 99.7, 101.6 (3 × d, C-1a, C-1b, C-1c), 101.7 (d, *C*HPh) and 170.3 (s, CONH).

Further elution gave the α anomer **7α** (238 mg) as a glass (Found: C, 67.70; H, 6.25; N, 2.10. $C_{74}H_{81}N_2O_{16}F_3$ requires C, 67.77; H, 6.23; N, 2.14%); $[\alpha]_D$ +18.2 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.38 (3 H, d, $J_{6a,5a}$ 6.0, H-6a), 1.77 (3 H, s, CH_3CO), 1.80–1.90 (2 H, m, OCH$_2$CH_2CH$_2$NHCOCF$_3$), 3.07 (1 H, dt, $J_{5c,6c}$ 10.0, $J_{5c,4c}$ 10.0, $J_{5c,6'c}$ 5.0, H-5c), 3.21 (1 H, br d, $J_{6b,6'b}$ 10.9, H6′b), 3.31–3.70 (10 H, m, H-3a, H-2b, H-6b, H-3c, H-4c, H-6′c, O(CH$_2$)$_2$CH_2NHCOCF$_3$, OCH_2(CH$_2$)$_2$-NHCOCF$_3$), 3.76–4.11 (7 H, m, H-2a, H-4a, H-5a, H-3b, H-4b, H-5b, H-6c), 4.26 (1 H, d, *J* 11.9, C*H*HPh), 4.48 (1 H, br s, H-1c), 4.50 (1 H, d, *J* 12.0, CH*H*Ph), 4.57–4.76 (9 H, m, 3 × C*H*HPh, 2 × CH_2Ph, H-1a, H-2c), 4.83 (1 H, d, C*H*HPh), 4.94 (1 H, d, $J_{1b,2b}$ 3.8, H-1b), 4.96 (1 H, d, *J* 11.0, CH*H*Ph), 4.98 (1 H, d, *J* 11.8, CH*H*Ph), 5.48 (1 H, s, C*H*Ph), 5.50 (1 H, d, *J* 9.7, NHAc), 6.75 (1 H, br s, NHCOCF$_3$) and 7.05–7.60 (35 H, m, 7 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 18.0 (q, C-6a), 23.1 (q, *C*H$_3$CO), 28.3 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 38.3 (t, O(CH$_2$)$_2$*C*H$_2$NHCOCF$_3$), 50.4 (d, C-2c), 65.8, 67.9, 68.6, 71.2, 72.2, 72.9, 73.5, 74.7, 75.3 (9 × t, C-6b, C-6c, 6 × *C*H$_2$Ph, O*C*H$_2$(CH$_2$)$_2$NHCOCF$_3$), 67.1, 68.6, 69.9, 74.9, 75.6, 75.9, 78.5, 79.0, 79.6, 80.0, 80.3 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.1, 97.7, 99.6 (3 × d, C-1a, C-1b, C-1c), 101.7 (d, *C*HPh) and 170.4 (s, CONH).

3-Trifluoroacetamidopropyl (2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 9α and its anomer 9β

A solution of **7α** (240 mg, 0.183 mmol) in CH_2Cl_2 (10 cm^3) was cooled to 0 °C, then 90% aq. CF_3COOH (2.5 cm^3) was added and the mixture was stirred for 30 min. The reaction was quenched with sat. $NaHCO_3$ and, after extraction with CH_2Cl_2, the organic layer was washed with sat. $NaHCO_3$ and water, dried (Na_2SO_4), filtered and concentrated. The crude diol **8α** was dissolved in toluene (30 cm^3) and Bu_2SnO (68 mg, 0.275 mmol) was added. The mixture was refluxed for 3 h, then concentrated to 1/3 of its volume and allowed to cool to 50–60 °C. Tetrabutylammonium iodide (102 mg, 0.275 mmol) and BnBr (65 mm^3, 0.549 mmol) were added and the mixture was refluxed overnight. The reaction was quenched with MeOH and concentrated. The residue was purified by flash chromatography (petroleum ether–EtOAc 6 : 4) to give **9α** (187 mg, 78%) isolated as a yellow glass (Found: C, 67.70; H, 6.40; N, 2.10. $C_{74}H_{83}N_2O_{16}F_3$ requires C, 67.67; H, 6.37; N, 2.13%); $[\alpha]_D$ +19.0 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.38 (3 H, d, $J_{6a,5a}$ 6.5, H-6a), 1.75 (3 H, s, CH_3CO), 1.84 (2 H, m, OCH$_2$CH_2CH$_2$-NHCOCF$_3$), 2.50 (1 H, br s, OH), 3.06–3.15 (2 H, m, H-5c, O(CH$_2$)$_2$CH*H*NHCOCF$_3$), 3.24–3.66 (11 H, m, H-3a, H-2b, H-6b, H-6′b, H-3c, H-4c, H-6c, H-6′c, O(CH$_2$)$_2$C*H*HNH-COCF$_3$, OCH_2(CH$_2$)$_2$NHCOCF$_3$), 3.77–3.90 (2 H, m, H-4a, H-5a), 3.93–4.13 (4 H, m, H-2a, H-3b, H-4b, H-5b), 4.23 (1 H, d, *J* 10.9, C*H*HPh), 4.37 (1H, d, *J* 11.9 CH*H*Ph), 4.49–4.81 (12 H, m, 9 × C*H*HPh, H-1a, H-1c, H-2c), 4.92–4.97 (4 H, m, H-1b, 3 × C*H*HPh), 5.73 (1 H, d, *J* 9.4, NHAc), 6.81 (1 H, br s, NHCOCF$_3$) and 7.15–7.50 (35 H, m, 7 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 18.1 (q, C-6a), 23.2 (q, *C*H$_3$CO), 28.4 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 38.3 (t, O(CH$_2$)$_2$*C*H$_2$NHCOCF$_3$), 49.4 (d, C-2c), 65.9, 68.2, 69.3, 71.0, 72.1, 72.8, 73.3, 73.6, 74.8, 75.3 (10 × t, C-6b, C-6c, 7 × CH$_2$Ph, O*C*H$_2$(CH$_2$)$_2$NHCOCF$_3$), 67.3, 68.6, 69.9, 75.2, 75.8, 79.1, 79.8, 80.0, 80.2, 80.7, 81.4 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.0, 97.7, 99.7 (3 × d, C-1a, C-1b, C-1c), 116.8 (q, $J_{C,F}$ 287.4, CO*C*F$_3$), 157.0 (q, $J_{C,F}$ 38.6, *C*OCF$_3$) and 170.4 (s, CONH).

The same reaction sequence was applied to compound **7β** (345 mg, 0.263 mmol) giving **9β** (272 mg, 76%) as a glass (Found: C, 67.70; H, 6.40; N, 2.10. $C_{74}H_{83}N_2O_{16}F_3$ requires C, 67.67; H, 6.37; N, 2.13%); $[\alpha]_D$ +39.4 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.40 (3 H, d, $J_{6a,5a}$ 6.2, H-6a), 1.72 (3 H, s, C*H*$_3$CO), 2.40 (1 H, br s, OH), 3.05–3.68 (14 H, m, H-3a, H-4a, H-5a, H-2b, H-6b, H6′b, H-3c, H-4c, H-5c, H-6c, H-6′c, OCH*H*$(CH_2)_2$NHCOCF$_3$, O$(CH_2)_2$C*H*$_2$NHCOCF$_3$), 3.72–3.81 (1 H, m, OCH*H*$(CH_2)_2$NHCOCF$_3$), 3.95–4.05 (2 H, m, H-3b, H-4b), 4.20–4.29 (2 H, m, H-2a, H-5b), 3.37–4.83 (14 H, m, H-1a, H-1c, H-2c, 11 × C*H*HPh), 4.88–5.02 (3 H, m, 3 × CH*H*Ph), 5.47 (1 H, d, *J* 3.7, H-1b), 5.62 (1 H, d, *J* 9.5, NHAc), 6.88 (1 H, br s, NHCOCF$_3$) and 7.10–7.50 (35 H, m, 7 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 17.8 (q, C-6a), 23.1 (q, *C*H$_3$CO), 28.7 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 37.2 (t, O$(CH_2)_2$*C*H$_2$NHCOCF$_3$), 49.4 (d, C-2c), 67.9, 68.4, 69.3, 71.0, 71.5, 72.1, 73.2, 73.5, 74.4, 75.4 (10 × t, C-6b, C-6c, 7 × *C*H$_2$Ph, O*C*H$_2$-$(CH_2)_2$NHCOCF$_3$), 67.4, 69.5, 72.3, 73.4, 75.1, 76.0, 79.4, 79.6, 80.2, 81.2 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.5, 99.8, 101.8 (3 × d, C-1a, C-1b, C-1c), 115.9 (q, $J_{C,F}$ 287.4, CO*C*F$_3$), 157.1 (q, $J_{C,F}$ 38.6, *C*OCF$_3$) and 170.3 (s, CONH).

3-Trifluoroacetamidopropyl (2-acetamido-3,6-di-*O*-benzyl-4-diphenylphospho-2-deoxy-β-D-mannopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside 10α and its anomer 10β

To a solution of **9α** (70 mg, 0.053 mmol) in dry CH_2Cl_2 (5 cm^3) were added Et(*i*-Pr)$_2$N (18 mm^3, 0.106 mmol) and DMAP (15 mg, 0.122 mmol) under N_2. The mixture was cooled to 0 °C, then diphenyl phosphorochloridate (25 mm^3, 0.122 mmol) was added. The reaction was stirred overnight at rt. Further portions of Et(*i*-Pr)$_2$N (18 mm^3, 0.106 mmol) and diphenyl phosphorochloridate (22 mm^3, 0.106 mmol) were added. After 30 h, the mixture was diluted with CH_2Cl_2, washed with ice–water, cold 0.5 M aq HCl, then with sat. $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated. The residue was purified by flash chromatography (petroleum ether–EtOAc 7 : 3) to give **10α** (40 mg, 49%) as a syrup (Found: C, 66.95; H, 6.05; N, 1.90. $C_{86}H_{92}N_2O_{19}PF_3$ requires C, 66.82; H, 6.00; N, 1.81%); $[\alpha]_D$ +26.4 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.35 (3 H, d, $J_{6a,5a}$ 6.5, H-6a), 1.72 (3 H, s, C*H*$_3$CO), 1.74–1.86 (2 H, m, OCH$_2$C*H*$_2$CH$_2$NHCOCF$_3$), 3.09 (1 H, m, H-5c), 3.21–3.69 (11 H, m, H-3a, H-2b, H-6b, H6′b, H-3c, H-6c, H-6′c, OC*H*$_2$-$(CH_2)_2$NHCOCF$_3$, O$(CH_2)_2$C*H*$_2$NHCOCF$_3$), 3.74–3.81 (2 H, m, H-4a, H-5a), 3.88–4.08 (4 H, m, H-2a, H-3b, H-4b, H-5b), 4.26 (1 H, d, *J* 11.0, CH*H*Ph), 4.28 (1 H, d, *J* 11.7, CH*H*Ph), 4.33 (1 H, d, *J* 12.2, CH*H*Ph), 4.41 (1 H, d, *J* 11.7, CH*H*Ph), 4.53–4.77 (12 H, m, H-1a, H-1c, H-2c, H-4c, 8 × C*H*HPh), 4.90–4.95 (3 H, m, H-1b, 2 × CH*H*Ph), 5.65 (1 H, d, *J* 9.7, NHAc), 6.75 (1 H, br s, NHCOCF$_3$) and 7.02–7.42 (45 H, m, 9 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 18.0 (q, C-6a), 23.1 (q, *C*H$_3$CO), 28.3 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 38.4 (t, O$(CH_2)_2$-*C*H$_2$NHCOCF$_3$), 49.4 (d, C-2c), 65.9, 68.0, 68.2, 70.6, 72.1, 72.8, 73.3, 73.4, 75.3 (10 × t, C-6b, C-6c, 7 × *C*H$_2$Ph, O*C*H$_2$-$(CH_2)_2$NHCOCF$_3$), 68.6, 69.9, 74.4, 74.6, 74.7, 76.1, 77.8, 79.0, 79.8, 80.0, 80.5 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.0, 97.7, 99.5 (3 × d, C-1a, C-1b, C-1c) and 170.5 (s, CONH); δ_P(200 MHz; $CDCl_3$, D_2O–H_3PO_4–H_2O = 90 : 8.5 : 1.5) −12.43.

The same procedure was applied to compound **9β** (50 mg, 0.038 mmol) giving **10β** (36 mg, 61%) as a syrup (Found: C, 66.95; H, 6.05; N, 1.90. $C_{86}H_{92}N_2O_{19}PF_3$ requires C, 66.82; H, 6.00; N, 1.81%); $[\alpha]_D$ +45.5 (*c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$, Me_4Si) 1.38 (3 H, d, $J_{6a,5a}$ 6.1, H-6a), 1.71 (3 H, s, C*H*$_3$CO), 1.60–1.80 (2 H, m, OCH$_2$C*H*$_2$CH$_2$NHCOCF$_3$), 3.02–3.15 (1 H, m, O$(CH_2)_2$C*H*HNHCOCF$_3$), 3.23 (1 H, br d, $J_{5c,4c}$ 9.0, H-5c), 3.28–3.48 (8 H, m, H-3a, H-5a, H-6b, H6′b, H-3c, H-6c, H-6′c, O$(CH_2)_2$C*H*HNHCOCF$_3$), 3.54–3.62 (3 H, m, H-4a, H-2b, OC*H*H$(CH_2)_2$NHCOCF$_3$), 5.72–3.79 (1 H, m, OC*H*H$(CH_2)_2$NHCOCF$_3$), 3.93–4.02 (2 H, m, H-4b, H-3b), 4.23–4.41 (6 H, m, H-1a, H-2a, H-5b, 3 × CH*H*Ph), 4.55–4.81 (11 H, m, H-1c, H-2c, H-4c, 8 × C*H*HPh), 4.90 (2 H, s, C*H*$_2$Ph), 4.97 (1 H, d, *J* 11.1, CH*H*Ph), 5.47 (1 H, d, *J* 3.7, H-1b), 5.71 (1 H, d, *J* 9.6, NHAc), 6.85 (1 H, br s, NHCOCF$_3$) and 7.01–7.45 (45 H, m, 9 × Ph); δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 17.8 (q, C-6a), 23.1 (q, *C*H$_3$CO), 28.7 (t, OCH$_2$*C*H$_2$CH$_2$NHCOCF$_3$), 37.2 (t, O$(CH_2)_2$*C*H$_2$NHCOCF$_3$), 49.4 (d, C-2c), 67.9, 68.0, 68.4, 70.6, 71.4, 72.1, 73.2, 73.4, 74.4, 75.3 (10 × t, C-6b, C-6c, 7 × *C*H$_2$Ph, O*C*H$_2$(CH$_2$)$_2$NHCOCF$_3$), 69.5, 72.3, 74.4, 74.6, 76.3, 77.9, 79.4, 79.6, 80.1, 81.2 (11 × d, C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-3c, C-4c, C-5c), 97.4, 99.6, 101.8 (3 × d, C-1a, C-1b, C-1c) and 170.4 (s, CONH); δ_P(200 MHz; $CDCl_3$, D_2O–H_3PO_4–H_2O = 90 : 8.5 : 1.5) −12.92.

3-Trifluoroacetamidopropyl (2-acetamido-2-deoxy-4-phospho-β-D-mannopyranosyl)-(1⟶4)-(α-D-glucopyranosyl)-(1⟶2)-α-L-rhamnopyranoside disodium salt 11α and its anomer 11β

Trisaccharide **10α** (40 mg, 0.026 mmol) was dissolved in MeOH–H_2O–EtOAc 3 : 2 : 2 (7 cm^3) and hydrogenolyzed at atmospheric pressure over $Pd(OH)_2$ (44 mg) for 16 h, then additional catalyst (45 mg) was added and the suspension stirred for another 40 h. The mixture was filtered over a Celite pad and the filtrate concentrated to dryness. The crude debenzylated product was dissolved in H_2O (2 cm^3) and further hydrogenolyzed over PtO_2 (15 mg) at 5 bar for 16 h. The reaction mixture was filtered over a Celite pad and the filtrate concentrated and eluted with water through a column filled with Dowex 50W-X8 resin (Na^+ form). Lyophilization gave 4c-*O*-phosphorylated trisaccharide **11α** (15 mg, 71%) as a white foam (Found: C, 37.20; H, 5.00; N, 3.55. $C_{25}H_{40}N_2O_{19}PF_3Na_2$ requires C, 37.23; H, 5.00; N, 3.47%); $[\alpha]_D$ +12.0 (*c* 1 in H_2O).

The same reaction sequence was applied to compound **10β** (36 mg, 0.023 mmol) affording trisaccharide **11β** (15 mg, 81%) as a white foam (Found: C, 37.20; H, 5.00; N, 3.55. $C_{25}H_{40}N_2O_{19}PF_3Na_2$ requires C, 37.23; H, 5.00; N, 3.47%); $[\alpha]_D$ +25.0 (*c* 1 in H_2O). NMR spectral data of compounds **11α** and **11β** are reported in Table 2.

3-Trifluoroacetamidopropyl (2-acetamido-2-deoxy-β-D-mannopyranosyl)-(1⟶4)-(α-D-glucopyranosyl)-(1⟶2)-α-L-rhamnopyranoside 12α and its anomer 12β

Trisaccharide **7α** (31 mg, 0.023 mmol) was dissolved in EtOAc–MeOH–H_2O 2 : 1 : 1 (8 cm^3) and hydrogenolyzed at atmospheric pressure over $Pd(OH)_2$ (30 mg) for 16 h, then additional catalyst (30 mg) was added and the suspension stirred for another 36 h. The mixture was filtered over a Celite pad and the filtrate lyophilised giving trisaccharide **12α** (14 mg, 89%) as a white foam (Found: C, 44.05; H, 6.00; N, 4.10. $C_{25}H_{41}N_2O_{19}F_3$ requires C, 43.99; H, 6.05; N, 4.10%); $[\alpha]_D$ = − 9.4 (*c* 1 in H_2O).

Trisaccharide **7β** (48 mg, 0.037 mmol) was hydrogenolyzed as described for its α anomer, affording trisaccharide **12β** (24 mg, 95%) as a white foam (Found: C, 44.05; H, 6.00; N, 4.10. $C_{25}H_{41}N_2O_{16}F_3$ requires C, 43.99; H, 6.05; N, 4.10%); $[\alpha]_D$ +17.9 (*c* 1 in H_2O); the NMR spectral data of compounds **12α** and **12β** are reported in Table 3.

3-Trifluoroacetamidopropyl (2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-hydrogen phosphonate-β-D-mannopyranosyl)-(1⟶4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside triethylammonium salt 13

To a stirred solution of **9α** (88 mg, 0.067 mmol) in dry CH_3CN (500 mm^3) and pyridine (300 mm^3) was added a 0.4 M solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one in CH_3CN (206 mm^3, 0.082 mmol) at rt. The mixture was stirred for 45 min, then py–H_2O 1 : 1 (500 mm^3) was added. The mixture was diluted with CH_2Cl_2, washed with H_2O and 1 M aq.

Table 2 ^{1}H NMR (D_2O, 500 MHz, 303 K) and ^{13}C NMR (D_2O, 125.72 MHz, 303 K) of compounds **11α** and **11β**[a]

	Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6′	NAc
11α	*a*	4.89	3.95	3.86	3.51	3.69	1.30		
		98.6	77.6	[70.9]	73.1	70.1	17.7		
	b	5.00	3.6	3.92	3.69	4.08	3.91	3.77	
		98.7	72.3	72.5	80.1	71.5	61.0		
	c	4.89	4.56	3.97	3.99	3.49	3.90	3.90	2.08
		100.8	53.6	73.4	70.8	77.4	61.7		23.2
	anchor	3.79	3.56	1.91	3.35	3.35			
		66.5		28.8	38.2				
11β	*a*	4.70	4.08	3.65	3.42	3.42	1.33		
		101.6	79.2	73.2	73.2	73.7	17.7		
	b	5.19	3.52	3.91	3.69	4.15	3.77	3.77	
		101.1	73.1	72.8	79.9	70.5	61.00		
	c	4.91	4.58	3.99	4.02	3.52	3.90	3.90	2.08
		100.6	53.9	73.1	71.8	77.1	61.6		23.2
	anchor	3.91	3.64	1.88	3.38	3.49			
		69.1		29.1	37.9				

[a] Chemical shifts referenced to the *N*-acetyl resonance at 2.083 and 23.23 ppm (compound **11α**) and to 3-trimethylsilyl-2,2,3,3-tetradeuteriopropanoic acid Na salt (TSP-d_4) at zero ppm and −1.8 ppm (compound **11β**). [ ] = uncertain assignment.

Table 3 ^{1}H NMR (D_2O, 500 MHz, 303 K) and ^{13}C- NMR (D_2O, 125.72 MHz, 303 K) of compounds **12α** and **12β**[a]

	Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6′	NAc
12α	*a*	4.89	3.96	3.85	3.50	3.67	1.30		
		98.7	77.7	71.0	73.3	70.2	17.7		
	b	5.00	3.58	3.91	3.70	4.09	3.77	3.79	
		99.0	72.4	72.6	80.0	71.6	60.9		
	c	4.90	4.56	3.84	3.54	3.47	3.94	3.82	2.08
		100.7	54.7	72.3	67.9	77.8	61.7		23.2
	anchor	3.80	3.56	1.91	3.45	3.45			
		64.9		27.1	36.6				
12β	*a*	4.71	4.08	3.66	3.43	3.41	1.33		
		101.6	79.3	73.3	73.7	73.3	17.7		
	b	5.19	3.53	3.91	3.70	4.15	3.77	3.77	
		101.1	73.1	72.8	79.9	71.5	61.0		
	c	4.90	4.56	3.82	3.53	3.46	3.94	3.83	2.08
		100.6	54.6	72.3	67.8	77.8	61.6		23.2
	anchor	3.90	3.64	1.89	3.40	3.50			
		68.2		29.1	37.9				

[a] Chemical shifts referenced to the *N*-acetyl resonance at 2.083 and 23.23 ppm.

triethylammonium hydrogen carbonate (TEAB) solution, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified on a short column of silica gel (CH_2Cl_2–MeOH 9 : 1 + 1% Et_3N) to give **13** (97 mg) as a syrup (Found: C, 65.00; H, 6.75; N, 2.80. $C_{80}H_{99}N_3O_{18}PF_3$ requires C, 64.98; H, 6.75; N, 2.84%). The formation of the H-phosphonate intermediate was ascertained by ^{1}H NMR analysis, which showed the diagnostic doublet at δ 6.95 ($J_{H,P}$ = 635 Hz). Compound **13** was used directly in the following coupling step without further characterization.

3-Trifluoroacetamidopropyl (2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl 4c-[(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl phosphate] triethylammonium salt 14

A mixture of **13** (97 mg, 0.066 mmol) and **5** (70 mg, 0.061 mmol) was dried by co-evaporation with pyridine (3 × 1 cm^3). The residue was dissolved in the same solvent (1 cm^3) and PivCl (19 mm^3, 0.153 mmol) was added. The mixture was stirred for 40 min at rt, then a freshly prepared solution of I_2 (31 mg, 0.122 mmol) in py–H_2O 19 : 1 (1 cm^3) was added. After 15 min the mixture was diluted with $CHCl_3$ and the phases were separated. The organic layer was washed with 1 M aq. $Na_2S_2O_3$ and 0.5 M aq. TEAB, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2–MeOH–Et_3N 97 : 2 : 1); the fractions containing the product were pooled and washed with 1 M aq. TEAB, and compound **14** (98 mg, 61%) was recovered as a glass (Found: C, 67.95; H, 6.60; N, 2.15. $C_{149}H_{172}N_4O_{33}PF_3$ requires C, 67.91; H, 6.58; N, 2.13%). ^{13}C and ^{31}P NMR analysis of compound **14** showed a mixture of α and β anomers at C-1d, causing extensive overlap of the signals in the ^{1}H NMR spectrum. Therefore, full ^{1}H NMR characterisation and optical rotation measurements were performed on deprotected dimer **15**. δ_C(75.46 MHz; $CDCl_3$, Me_4Si) 8.4 (q, NCH_2CH_3), 18.0, 18.1 (2 × q, C-6a, C-6d), 23.2 (q, CH_3CO), 28.3 (t, $OCH_2CH_2CH_2NHCOCF_3$), 38.3 (t, $O(CH_2)_2CH_2NHCOCF_3$), 45.3 (t, NCH_2CH_3), 50.5 (d, C-2c, C-2f), 65.8, 68.1, 68.7, 70.7, 71.2, 71.7, 71.9, 72.8, 73.4, 74.5, 74.6, 74.8, 74.9, 75.2 (14 × t, C-6b, C-6c, C-6e, C-6f, 13 × CH_2Ph, $OCH_2(CH_2)_2NHCOCF_3$), 67.1, 68.5, 69.3, 69.6, 70.4, 71.4, 74.3, 75.5, 76.0, 76.2, 78.6, 78.8, 79.4, 79.8, 80.0, 80.3, 80.7, 81.1, 81.3, 81.5 (20 × d, C-2a, C-2b, C-2d, C-2e, C-3a, C-3b, C-3c, C-3d, C-3e, C-3f, C-4a, C-4b, C-4c, C-4d, C-4e, C-4f, C-5a, C-5b, C-5c, C-5d, C-5e, C-5f), 94.0, 96.2, 96.7, 97.6, 98.9, 99.6, 101.6 (7 × d, C-1a, C-1b, C-1c, C-1d, C-1e, C-1f, *C*HPh) and 170.1, 170.4 (2 × s, 2 × CONH); δ_P(200 MHz; $CDCl_3$, D_2O–H_3PO_4–H_2O = 90 : 8.5 : 1.5) −2.46 (major isomer), −1.09 (minor isomer).

Table 4 ^{1}H NMR (D_2O, 500 MHz, 303 K) and ^{13}C NMR (D_2O, 125.72 MHz, 303 K) of compounds **15α**[a]

Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6	H-6′	NAc
a	4.86	3.925	3.82	3.48	[3.88]	1.27		
	98.3	77.3	70.6	72.8		17.5		
b	5.00	3.56	3.87	3.68	4.07	3.75	[3.75]	
	98.7	72.1		79.7	71.3	60.7		
c	4.89	4.57	3.98	4.05	3.54	3.83	[3.91]	[2.06]
	100.2	54.2	72.2	73.1	76.5	61.3		[23.0]
d	5.48/5.49	4.01	3.92	3.49	3.87	1.29		
	94.8	77.8	70.0	72.8		17.6		
e	4.96	3.55	3.88	3.67	4.05	3.75	[3.75]	
	98.5	72.1		79.7	71.3	60.7		
f	4.87	4.53	3.81	3.51	3.44	3.81	[3.91]	[2.06]
	100.2	54.3	73.1	67.5	77.5	61.3		[23.0]
anchor	3.76	1.88	3.42					
	3.53	1.88						

[a] Chemical shifts referenced to the *N*-acetyl resonance at 2.083 and 23.23 ppm. [ ] = uncertain assignment.

3-Trifluoroacetamidopropyl (2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl 4c-[2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl phosphate] triethylammonium salt 15

Compound **14** (41 mg, 0.015 mmol) was hydrogenolyzed over $Pd(OH)_2$ (40 mg) in MeOH–H_2O 1 : 1 (3 cm^3) for 4 days at rt (addition of a further 20 mg of catalyst was required to drive the reaction to completion). The mixture was filtered over a Celite pad and the filtrate was concentrated, then eluted with water through a column filled with Dowex 50W-X8 resin (Na^+ form). The fractions containing the dimer were pooled and lyophilized to give **15** (18 mg, 96%) as a white solid (Found: C, 41.75; H, 5.70; N, 3.25. $C_{45}H_{74}N_3O_{33}PF_3Na$ requires C, 41.70; H, 5.75, N, 3.24%). The diastereomeric ratio of α : β anomers of **15** was determined to be 9 : 1 by HPLC analysis, performed with a column of porous graphitised carbon (PGC "Hypercarb®", Hypersil, Runcorn, UK; 4.6 × 250 mm, 7 μ)[21] eluted with an acetonitrile gradient (2 to 25%) in purified water (Elga) containing 0.05% TFA at 0.6 cm^3 min^{-1}. Elution was monitored by UV at 206 nm. $[\alpha]_D$ +24.0 (*c* 1 in H_2O); the NMR spectral data of compound **15α** are reported in Table 4.

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